

**Amendment to the Claims:**

This listing of Claims will replace all prior versions, and listings, of claims in the application.

**Listing of Claims:**

Claims 1-16 (cancelled).

Claim 17 (currently amended): 17. A method for detecting a nucleic acid target sequence in a sample, comprising the steps of:

(a) obtaining a sample of nucleic acid material to be analyzed;

(a)(b) ~~forming~~ selecting a nucleic acid target sequence having a length of up to 130 nucleotides expected to be found in said nucleic acid material;

(b)(c) ~~forming~~ synthesizing a primer pair for amplifying said target sequence in a polymerase chain reaction to produce a double stranded amplification product, said primer pair including a forward primer and a reverse primer;

(c)(d) preparing a dye for labeling said double stranded amplification product in solution when such amplification product is formed said dye being detectable by a fluorimetric measuring device;

(d)(e) introducing a quantity of said forward and reverse primers, a quantity of thermostable nucleic acid polymerase, a quantity of deoxynucleotide triphosphates, a quantity of

said dye, and a quantity of said nucleic acid material containing said target sequence in an aqueous reaction medium;

(e)(f) inducing an amplification reaction, producing a quantity of said double stranded amplification product derived from said target sequence, said dye thereafter labeling said amplification product; and

(f)(g) detecting said labeled amplification product by detecting said dye with said fluorimetric a measuring device.

Claims 18 and 19 (cancelled).

Claim 20 (new): 20. The method of claim 17, wherein said nucleic acid target sequence has a length in the range of from about 25 nucleotides to about 100 nucleotides.

Claim 21 (new): 21. A method for detecting a nucleic acid target sequence in a sample, comprising the steps of:

- (a) obtaining a sample of nucleic acid material to be analyzed;
- (b) selecting a nucleic acid target sequence expected to be found in said nucleic acid material;

- (c) synthesizing a primer pair for amplifying said target sequence in a polymerase chain reaction, said primer pair including a forward primer and a reverse primer;
- (d) providing a first dye and a second dye, the first dye and second dye being of the type which are capable of interacting to product a signal detectable by a fluorimetric measuring device when said first dye is positioned within a specific proximity of said second dye and the two dyes are subjected to an external energy stimulus;
- (e) labeling said forward primer with said first dye to form a labeled forward primer;
- (f) labeling said reverse primer with said second dye to form a labeled reverse primer;
- (g) providing a quantity of said labeled forward primer and a quality of said labeled reverse primer, a quantity of thermostable nucleic acid polymerase, a quantity of deoxynucleotide triphosphates, and a quantity of said nucleic acid material in an aqueous reaction medium, in a reaction vessel;
- (h) initiating a polymerase chain reaction in said reaction vessel to amplify said target sequence, said polymerase chain reaction producing a first quantity of double stranded amplification product of said target sequence, said amplification product incorporating said labeled forward primer on a first strand of said amplification product and incorporating said

labeled reverse primer on the opposite complementary strand of said amplification product, said labeled forward and labeled reverse primers located within said specific proximity such that said first dye of said labeled forward primer and said second dye of said labeled reverse primer interact to produce a signal on application of said external energy stimulus, detectable by a fluorimetric measuring device.

Claim 22 (new). 22. The method of claim 21, wherein said first and second dyes are fluorescent dyes.

Claim 23 (new). 23. The method of claim 21, wherein said first and second dyes include phosphorescent dye moieties.

Claim 24 (new). 24. The method of claim 21, wherein said first and second dyes include luminescent dye moieties.

Claim 25 (new). 25. The method of claim 21, wherein said nucleic acid material includes double stranded DNA.

Claim 26 (new). 26. The method of claim 21, wherein said nucleic acid polymerase is *Thermus aquaticus* (Taq) polymerase.

Claim 27 (new). 27. The method of claim 21, wherein said nucleic acid polymerase is Pfu polymerase.

Claim 28 (new). 28. The method of claim 21, wherein said signal involves fluorescent resonance energy transfer.

Claim 29 (new). 29. The method of claim 21, wherein said specific proximity is not greater than about 100 Å°.

Claim 30 (new) 30. The method of claim 21, wherein said measuring device is a spectrofluorimeter.

Claim 31 (new) 31. The method of claim 21, wherein said target sequence has a length of up to about 130 nucleotides.

Claim 32 (new). 32. The method of claim 21, wherein said target sequence has a length in the range of about 25 nucleotides to about 100 nucleotides.

Claim 33 (new). 33. The method of claim 21, wherein said amplification product has a length of no greater than about 130 base pairs.

Claim 34 (new). 34. The method of claim 21, wherein said first dye is a donor fluorophore and said second dye is an acceptor fluorophore, and wherein said application of said energy stimulus produces fluorescent resonance energy transfer (FRET) between said first dye and said second dye, and said signal detected by said fluorimetric measuring device is a fluorescent emission signal produced by said second dye following FRET.

Claim 35 (new). 35. The method of claim 21, wherein a control reaction is completed, using de-ionized water in place of said nucleic acid material, establishing a background signal level detected by said measuring device which may be compared to said signal produced by said amplification product.

Claim 36 (new) 36. The method of claim 35, further comprising the steps of:

applying said external energy stimulus throughout at least a portion of the duration of said polymerase chain reaction; and

while applying said external energy stimulus, monitoring the intensity of said signal produced by said amplification product against said background signal level as a function of time to determine the concentration of said amplification product on a real time basis.

Claim 37 (new) 37. The method of claim 21, comprising an additional step of conducting a melting temperature analysis of said amplification product produced by said step of initiating a polymerase chain reaction.

Claim 38 (new). 38. The method of claim 21, wherein said sample of nucleic acid material includes messenger RNA (mRNA), and said nucleic acid target sequence is a nucleotide sequence of said mRNA, and wherein said step of initiating a polymerase chain reaction includes a first cycle that includes annealing said labeled forward primer to said mRNA target sequence, and using said labeled forward primer to initiate production of a complementary strand of DNA (cDNA) from said template mRNA strand, and includes further cycles amplifying said double stranded cDNA and mRNA product to produce a double stranded amplification product incorporating said labeled forward and labeled reverse primers within said specific proximity and producing said signal on application of said energy stimulus, detectable by said fluorimetric measuring device.

Claim 39 (new). 39. The method of claim 21, wherein said steps of synthesizing said primer pair and labeling said forward primer and said reverse primer includes structuring the nucleotide

sequences of each of said primers such that said labeled forward primer hybridizes to a first nucleotide sequence flanking said nucleic acid target sequence on a first strand of nucleic acid material, and said labeled reverse primer hybridizes to a second nucleotide sequence flanking said target sequence on the opposite complementary strand, said labeled forward and reverse primers being incorporated into said amplification product on opposite strands and flanking said target sequence with said first and second dyes within said specific proximity, such that said signal intensity is analyzed to determine the length of said target sequence.

Claim 40 (new) 40. The method of claim 21 comprising the additional steps of:

- (a) selecting a second nucleic acid target sequence expected to be found in said sample of nucleic acid material;
- (b) synthesizing a second primer pair for amplifying said second nucleic acid target sequence, said second primer pair including a second forward primer and a second reverse primer;
- (c) providing a third dye and fourth dye, said third dye and said fourth dye being of the type which are capable of interacting to produce a second signal detectable by said measuring device when said third dye is positioned within a second specific proximity of said fourth dye, and the two dyes are subjected to a second external energy stimulus;
- (d) labeling said second forward primer with said third dye to produce a labeled second forward primer;

(e) labeling said second reverse primer with said fourth dye to produce a labeled second reverse primer;

(f) at the time of said first step of introducing, second introducing a quantity of said labeled second forward primer and a quantity of said labeled second reverse primer in said aqueous reaction medium in said reaction vessel; and

(g) at the time of said first step of initiating, second initiating a second polymerase chain reaction to amplify said second nucleic acid target sequence producing a first quantity of a second double stranded amplification product of said second nucleic acid target sequence, said second amplification product incorporating said labeled second forward primer and said labeled second reverse primer on opposite complementary strands of said second amplification product, and said third dye and said fourth dye located within said second specific proximity and producing a second signal on application of said second energy stimulus, detectable by said fluorimetric measuring device, permitting detection of said first and second nucleic acid target sequences in the same reaction process.

Claim 41 (new) 41. The method of claim 40, wherein said first target sequence includes a first mutation point and said second target sequence includes a second mutation point, enabling the user to identify two mutations in said sample in the same reaction process.

Claim 42 (new) 42. The method of claim 41, wherein said first amplification product and said second amplification product are subjected to melting, and the melting points of the first



amplification product and the second amplification product are analyzed to identify the original first and second target sequences in said sample.

Claim 43 (new). 43. The method of claim 42, wherein, during said melting analysis, and as the first and second amplification products are melted, the relative intensity of the signal of each amplification product is monitored during melting in order to determine the quantity of original first and second target sequences in said sample.

Claim 44 (new). 44. The method of claim 40, wherein said first target sequence and said second target sequence are the same except that the second target sequence includes a mutation in at least one nucleotide of its sequence.

Claim 45 (new) 45. The method of claim 40, comprising the additional steps of forming a plurality of labeled primer pairs, each primer pair designed to target a different target sequence and producing a different signal detectable by said measuring device on amplification and incorporation of said primer pairs into the double stranded amplification product produced from the respective target sequence, enabling the user to identify multiple target sequences in said sample in the same reaction process.

Claim 46 (new). 46. The method of claim 45, wherein said polymerase chain reaction is conducted at a temperature of a sufficiently high level that only selected thermally stable amplification products will survive and be available for analysis of their respective signals by said measuring device.

Claim 47 (new). 47. A method for detecting a nucleic acid target sequence and a variant of said nucleic acid target sequence containing a mutation point expected to be found in a sample of nucleic acid material, comprising the steps of:

- (a) obtaining a sample of nucleic acid material to be analyzed;
- (b) identifying a nucleic acid target sequence expected to be found in said nucleic acid material;
- (c) identifying a variant of said nucleic acid target sequence (the variant target sequence) expected to be found in said sample of nucleic acid material;
- (d) synthesizing a primer triplet for amplifying said nucleic acid target sequence and said variant target sequence in a polymerase chain reaction to produce two different double stranded amplification products, said primer triplet including a first forward primer designed to hybridize to the 3' end of the variant target sequence, a second forward primer designed to hybridize to the 3' end of the nucleic acid target sequence, and a reverse primer; the reverse primer designed to hybridize to the complementary strand of both the target nucleic acid sequence and variant target sequence;
- (e) labeling said reverse primer with a donor dye moiety, to form labeled reverse primer;
- (f) labeling said first forward primer with a first acceptor dye moiety, to form labeled first forward primer, said donor dye moiety and said first acceptor dye moiety being of a type which are capable of interacting to produce a signal detectable by a fluorimetric measuring

device when said donor dye moiety is positioned within a specific proximity of said first acceptor dye moiety and the donor and first acceptor dye moieties are subjected to an external energy stimulus;

(g) labeling said second forward primer with a second acceptor dye moiety, forming a labeled second forward primer, said donor dye moiety and said second acceptor dye moiety being of a type which are capable of interacting to produce a second signal detectable by said measuring device when said donor dye moiety is positioned within a second specific proximity of said second acceptor dye moiety and the donor and second acceptor dye moieties are subjected to an external energy stimulus;

(h) providing a quantity of said labeled first forward primer, a quantity of said labeled second forward primer, and a quantity of said labeled reverse primer, a quantity of thermostable nucleic acid polymerase, a quantity of deoxynucleotide triphosphates, and a quantity of said sample of nucleic acid material in an aqueous reaction medium, in a reaction vessel; and

(i) initiating a polymerase chain reaction in said reaction vessel to amplify said nucleic acid target sequence and said variant target sequence, said polymerase chain reaction producing a first amplification product derived from said variant target sequence, said first amplification product incorporating said labeled first forward primer and said labeled reverse primer, and a second amplification product derived from said non-mutated target sequence, said second amplification product incorporating said labeled second forward primer and said labeled reverse primer, such that the first acceptor dye moiety of said first forward primer and the donor

dye moiety of said reverse primer are positioned within said specific proximity and interact on application of said first energy stimulus to produce said first signal detectable by said fluorimetric measuring device, and said second acceptor dye moiety of said labeled second forward primer and said donor dye moiety of said labeled reverse primer are positioned within said second specific proximity and interact on application of said second energy stimulus to produce said second signal detectable by said fluorimetric measuring device, permitting the identification of said nucleic acid target sequence and said variant target sequence in the same reaction process.

Claim 48 (new). 48. The method of claim 47, wherein said variant target sequence includes a single nucleotide polymorphism.

Claim 49 (new). 49. The method of claim 47, wherein said variant target sequence includes at least one insertion/deletion mutation.

Claim 50 (new). 50. The method of claim 47, comprising the further steps of:

following determination of a background signal level of the materials provided in said reaction vessel, except for said sample, through an initial control reaction, applying first and second external energy stimuli throughout at least a portion of the duration of said polymerase chain reaction; and

while applying said first and second external energy stimuli, monitoring the intensity of said first and second signals against said background signal level as a function of time to

determine the relative concentrations of said first amplification product and said second amplification product on a real-time basis.

Claim 51 (new). 51. The method of claim 40 comprising the further steps of:

following determination of a background signal level of the materials introduced in the reaction vessel except for said sample through an initial control reaction, applying first and second external energy stimuli throughout at least a portion of the duration of said polymerase chain reaction; and

while applying said first and second external energy stimuli, monitoring the intensity of said first and second signals against said background signal level as a function of time to determine the relative concentrations of said first amplification product and said second amplification product on a real time basis.

Claim 52 (new). 52. A method for analyzing nucleic acid samples for polymorphisms, comprising the steps of:

- (a) obtaining a sample of nucleic acid material to be analyzed:
- (b) selecting at least a first nucleic acid target sequence and a second nucleic acid target sequence, a portion of said first nucleic acid target sequence overlapping a portion of said second nucleic acid target sequence;
- (c) synthesizing a first primer pair for amplifying said first nucleic acid target sequence to form a first amplification product, said first primer pair including a first forward primer and a first reverse primer;

(d) synthesizing a second primer pair for amplifying said second nucleic acid target sequence to form a second amplification product, said second primer pair including a second forward primer and a second reverse primer;

(e) obtaining a dye that binds to double stranded nucleic acid amplification products of the type that includes said first amplification product and said second amplification product, and that, after binding to said amplification products, and on application of an external energy stimulus, emits a signal detectable by a measuring device;

(f) providing a quantity of said first primer pair, a quantity of said second primer pair, a quantity of said sample of nucleic acid material, a quantity of thermostable nucleic acid polymerase, and a quantity of deoxynucleotide triphosphates, in an aqueous reaction medium, in a reaction vessel;

(g) initiating a polymerase chain reaction in said reaction vessel to amplify said first target sequence to form said first amplification product and to amplify said second target sequence to form said second amplification product; and

(h) with said measuring device, monitoring the formation of said first and second amplification products by applying an energy stimulus to said reaction medium to detect said signal indicating the presence of said amplification products; and

(i) performing an analysis of said amplification products formed in said polymerase chain reaction, to determine the identity of each said amplification product.